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Hematopoietic Stem Cell Transplantation for Severe Combined Immunodeficiency Diseases

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ABSTRACT

Hematopoietic stem cell transplantation (HSCT) is the only curative option for most children with severe combined immunodeficiency disease (SCID). Survival for SCID following HSCT has significantly improved over the past several decades, and ranges from 70% to 95% depending on the clinical condition of the child at the time of transplant, the availability of an HLA-matched sibling donor, and the SCID genotype/phenotype. In this article we will review the types of SCID and discuss the critical HSCT issues that confront us today, including the optimal source of donor cells when an HLA-matched sibling is not available, as well as the pros and cons of using conditioning therapy pretransplant. As SCID children have been followed for several decades, it is becoming apparent that long-term outcome and durable T and B cell immune reconstitution are quite variable depending on the initial treatment and source of donor cells. Finally, the development of methods to improve the early diagnosis of SCID along with designing prospective trials to evaluate the best approaches to curing these diseases with minimal toxicity are critical to improving outcomes for children with SCID.

KEY WORDS

Severe combined immunodeficiency (SCID) • Hematopoietic stem cell transplantation. Immune reconstitution • Newborn screening • Public health • Primary immunodeficiency • Genetic disease • Early diagnosis

INTRODUCTION

Although early results using gene therapy for some types of severe combined immunodeficiency diseases (SCID) are encouraging [1,2], hematopoietic stem cell transplantation (HSCT) remains the only curative option for most children with SCID. There has been a dramatic improvement in outcomes since the first transplants were done about 40 years ago, mostly as a result of significant advances in HLA typing, early recognition and diagnosis, genotyping, detection, and overall supportive care including detection and treatment of opportunistic infections. Today, children with SCID who are healthy at the time of transplant, who are recipients of HLA-matched donor transplants, and who do not require conditioning therapy can expect a better than 90% chance of long-term disease-free survival [2,3].

The major issues that confront us today involve (1) the durability of the stem cell graft and subsequent immune reconstitution, (2) donor selection when an HLA-matched sibling is not available and the type, and (3) intensity of conditioning regimens if one is necessary. With respect to the latter, an additional concern is potential long-term effects of conditioning therapy on these very young infants. As newborn screening for SCID becomes a reality, these issues become even more critical. Virtually all of the information that we have today regarding short- and long-term outcomes for children with SCID is the result of retrospective reports from either

single institutions or cooperative groups (EBMT or CIBMTR). The need for prospective randomized studies to evaluate some of these critical issues for children with SCID is obvious.

SCID GENOTYPES AND PHENOTYPES

Many genetic mutations involving critical proteins in DNA synthesis, T cell signaling, or V(D)J recombination have been identified as causing SCID [4]. These mutations result in several distinct phenotypes based upon the presence or absence of T, B, and NK cells. The most common cause of SCID results from mutations in the ILγc gene, and is seen in about 50% of all SCID cases. Generally, these children have the $T^-B^+NK^-$ phenotype, and presumably, because of the lack of NK cells, have durable T cell reconstitution without any conditioning therapy [5].

It is estimated that 20% of SCID is associated with the $T^-B^-NK^+$ phenotype, most commonly seen with a mutation in the RAG1 or RAG2 genes [6]. Another group of patients with $T^-B^-NK^+$ SCID is associated with mutations in genes coding for proteins involved in the nonhomologous DNA repair pathway, Artemis and Ligase IV [7-9]. Cells from these patients show increased sensitivity to ionizing radiation and alkylating agents. There is a high incidence of SCID secondary to a single point mutation in the gene that codes for Artemis among Athabascan speaking Native Americans in the Southwestern United States, in particular the Navajo and Apache Indians [8]. At least 1 study suggests that Navajo Indian children with SCID are particularly susceptible to treatment with alkylating agents, especially with respect to subsequent growth and development [10]. There is also at least 1 report of children with Artemis mutations who presented with EBV-related lymphomas, suggesting an increased susceptibility to cancer in this patient population [11]. Interestingly, these patients had incomplete Artemis mutations and a leaky SCID phenotype with some B cells present.

The other SCID phenotypes ($T^-B^+NK^+$ and $T^-B^-NK^-$) represent most of the remaining causes with a variety of defects that include ADA and PNP deficiency, IL7Rα defects, and defects in CD3δ, ϵ, and ζ, among others. There remains a less well-characterized group of SCID patients who present with T, B, and NK cells. Some of these patients have leaky mutations in genes such as RAG1/2 (Omenn's syndrome), Artemis, or ILγc; some are engrafted with maternal T cells, whereas the rest have no identified genotype to date.

ENGRAFTMENT AND IMMUNE RECONSTITUTION POST-HSCT

Because children with SCID have by definition absent to very low T cell immunity, it would be expected that they would be unlikely to reject a hematopoietic stem cell graft. There are 3 conditions in which engraftment and reconstitution of at least T cell immunity is likely to occur in this patient population even without immunosuppressive conditioning therapy: (1) when an HLA-matched related donor is available, (2) when maternal cells have engrafted in utero, or (3) when NK cells are absent. NK cells are thought to play a major role in graft resistance in children with SCID undergoing a haplocompatible transplant. However, regardless of the SCID phenotype, stem cells from HLA-matched sibling readily engraft without conditioning.

There are, however, reports of a gradual loss of T cell immunity based on thymic T cell receptor gene excision circle (TREC) output as well as T cell receptor diversity in patients with SCID who have not received conditioning [5,12]. This has led to some centers advocating the use of ablative therapy for all children with SCID undergoing a HSCT regardless of the phenotype or genotype. Of course, when unrelated donors are used, either adult volunteers or umbilical cord blood, ablative conditioning has generally been the rule [3,13]. The situation is further complicated by the fact that a subgroup of SCID patients have DNA repair defects that make them particularly susceptible to alkylating agents and ionizing radiation [10]. The paucity of data examining the late effects of conditioning on this particular patient population, most of whom are treated within the first year of life, further complicates the debate over the optimal approach for therapy.

ALTERNATIVE DONORS

When an HLA-matched related donor is not available there are 3 alternative sources that can be used: a haplocompatible relative, HLA-matched unrelated adult volunteer, or an unrelated banked UCB unit. Although most of the experience with alternative donors has been with haplocompatible relatives, there is a growing use of unrelated donors, either adult volunteers or cord blood [3,13,14]. There are advantages and disadvantages to each alternative donor source and unfortunately, because all of the reports to date have been historic retrospective comparisons it is virtually impossible to determine which approach might be optimal. Almost all of the transplants using unrelated donors have employed either full myeloablative or reduced intensity RIC regimens, whereas many of the haplocompatible donor studies have not used conditioning, at least as the initial transplant approach. This could certainly affect results of engraftment efficiency, T and B cell reconstitution, and late effects. The major concerns regarding the use of haplocompatible donors are the rejection rate, the relative delay in T cell recovery, and the loss of naïve

T cells and normal thymopoiesis over time when conditioning is not used. Newer technologies for stem cell mobilization and efficient T cell depletion (TCD) have resulted in the ability to administer much larger doses of $CD34^+$ cells to SCID patients, which could favorably impact at least some of these issues.

CONDITIONING VERSUS NO CONDITIONING

The majority of children with SCID should not theoretically need conditioning to overcome graft resistance regardless of the HLA compatibility of the donor; 50% have NK^- SCID and of those remaining, 30% to 40% are maternally engrafted, and ~20% will have a matched sibling. For the remaining children, engraftment without immunosuppressive therapy may still be possible. For example, a perfectly matched unrelated donor (10 of 10 allele match) may engraft without conditioning just like a genotypic matched sibling [13]. In our own experience (unpublished), using megadoses of haplocompatible T cell-depleted $CD34^+$ PBSCs, about 40% of patients with NK^+ SCID will engraft and reconstitute T cell immunity.

The advantages of using no chemotherapy in these children who are generally <6 months of age are obvious; late effects on growth, development, and organ function will be minimized. However, the majority of recipients of HSCT who do not receive any kind of myelosuppressive conditioning do not reconstitute B cell immunity, and are required to be on gammaglobulin therapy for the rest of their lives. This in and of itself might be an acceptable tradeoff in terms of the toxic effects of chemotherapy. However, the reports of incomplete T cell reconstitution in recipients of RIC transplants, compared to those who receive myeloablation, with a gradual loss of T cell immunity over time require us to consider this approach more carefully [5,12]. Unfortunately, there has been little correlation of these changes in T cell immunity with clinical outcome, and it may require longer follow-up to determine the exact implications.

One way to address the toxic side effects of conditioning in a young infant would be to do an initial transplant without conditioning to restore T cell immunity, followed by a second transplant using the same donor and ablative conditioning to establish a B cell graft and durable T cell immunity once the patient is older. Of course, this would not work for unrelated cord blood transplants, and would be logistically challenging when using unrelated volunteer donors. It is clear that what is needed is a detailed long-term follow-up study of a large number of children with SCID stratified by phenotype, genotype, and donor source that compares those who received conditioning (immunosuppressive and/or myeloablative) to those who did not. Ideally, this would be followed by a prospective study that is designed based upon the results of the retrospective analysis.

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Late Immunologic and Clinical Outcomes for Children with SCID

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SCID comprises a heterogeneous group of genetic disorders that are characterized by profound impairment of T lymphocyte differentiation always associated with a direct or indirect deficiency in B cell immunity. Consequently, in the absence of treatment, patients are highly susceptible to infection and usually die within the first year of life. Allogeneic hematopoietic stem cell transplantation (HSCT) represents a life-saving treatment of these conditions.

As discussed above, a unique aspect of HSCT in SCID is that myeloablative conditioning is not required for donor cell engraftment. This situation offers a significant clinical advantage, as toxicity of the procedure can be reduced. This favorable setting also led to the use of haploidentical parental HSCT for the many SCID patients who do not have an HLA-identical sibling. This development was made possible by the availability of techniques allowing for depletion of mature T cells from the graft.

The overall survival (OS) rate of SCID patients who have undergone an HSCT from an HLA-identical sibling is good, being over 80% in patients treated from 1968 until now [1,2]. With improvements in the treatment of severe infection, the survival rate is over 90% since 1996 [1]. The survival rate of patients treated by a haploidentical T cell-depleted (TCD) HSCT is not as favorable with long-term survival ranging from 50 to 78% [1,2]. Survival rates have, however, increased over time from 35% survival in patients transplanted before 1985 to 75% in those treated between 1996 and 1999 [1]. These superior outcomes are because of, in large part, more effective prevention and treatment of disease-related and procedure-related complications (infections and graft-versus-host disease [GVHD]). Several parameters other than donor availability play a role in determining survival: age at diagnosis, clinical status at diagnosis, occurrence of GVHD, graft rejection, and kinetics of T cell reconstitution. For example, patients transplanted during the first 3.5 months of life have a better survival rate compared to patients transplanted after that age, regardless of the type of donor [2,3]. Despite TCD of the graft in haploidentical HSCT, acute and chronic GVHD (aGVHD, cGVHD) still significantly have an impact on survival [1] . Graft rejection appears to be an issue for patients with the NK^+ type of SCID; haploidentical HSCT is associated with an increased rate of graft failure and a poorer prognosis for patients with this SCID subset. It was recently shown that only 35% of NK^+B^- SCID patients were long-term survivors versus 60% of NK^-B^+ SCID patients [4]. Patterns of T cell reconstitution following HLA-identical and haploidentical HSCT are very different, and also affect outcomes. In HLA-identical HSCT, the graft consists of progenitor cells as well as mature T cells. Therefore, T cell reconstitution is bimodal, with an early expansion of mature T cells followed by a second wave of naive T cells, 3 to 4 months after HSCT, that results from neothymopoiesis [3]. In haploidentical HSCT, only selected hematopoeitic progenitors are injected. Thus, the absence of early mature T cell expansion results in a prolonged T cell immunodeficiency during the 3 to 4 months post-HSCT.

In the absence of myeloablative conditioning, there is a split chimerism in most SCID patients (>80%), with T cells and NK cells being of donor origin in patients with a T^-NK^- type of SCID [5]. All other leukocyte subsets as well as hematopoietic lineages are of host origin. T cell progenitors, committed pro-T cells, common lymphoid progenitors, multipotent progenitors, or HSCs of donor origin migrate to and colonize the thymus early after HSCT. A wave of T cell differentiation ensues, which is sufficient to (re)populate all T-lymphocyte niches and account for the development of efficient T cell immune responses [3,5,6]. There is, however, no engraftment of stem cells in the bone marrow.

What are the consequences of the absence of stem cell engrafment on long-term immune reconstitution? This model predicts that after several years, the potential for T cell lymphopoiesis is exhausted, leaving the patient with a given set of T cells for the remainder of his/her life. This finding was first shown by Patel et al. [7], by quantifying TRECs in circulating T cells sequentially after HSCT. The presence of TRECs denotes that the T cells have not undergone division following TCR gene rearrangement, and thus detects naïve T cells. Such $TREC^+$ T cells are no longer detectable 10-12 years after HSCT performed in SCID patients without myeloablative therapy [7]. There is an alternative explanation for this unequivocal fall in naïve T cell counts: because the thymus in an SCID patient developments in the absence of functional

T cell precursors, it might not be able to support thymopoiesis as well as a healthy (non-SCID) thymus. In addition, thymi in SCID patients may be further damaged by infectious events or GVHD [8]. In our experience, the failure of secondary haploidentical HSCT to improve T cell immunity, when performed several years after the first HSCT, is consistent with the latter hypothesis.

To further assess the significance of $TREC^+$ T cell detection after HSCT in SCID patients, we have attempted to correlate quantitation of $TREC^+$ T cells with chimerism [9]. Within a group of patients who received HSCT >10 years ago, there is a strong correlation between detection of $TREC^+$ T cells and donor myeloid chimerism; virtually no $TREC^+$ T cells were detected in the absence of myeloid donor chimerism, whereas $TREC^+$ T cells were found, albeit in variable numbers, in patients with evidence for donor myeloid cells. Of note, we also found a correlation between the use of myeloablative conditioning regimen and myeloid chimerism. These data strongly support the concept that when donor myeloid cells are present, as a marker of donor-derived hematopoiesis, thymopoiesis does persist, even if the thymus is not entirely normal. Hence, it is possible that thymic function in SCID patients is lost when donor progenitor cells, which have emigrated to it, are exhausted. If true, this concept implies that everything possible should be done to maintain uninterrupted thymopoiesis in treated SCID patients. Persistence of naïve T cells was also positively correlated with overall T cell reconstitution, and as expected, a strong correlation between detection of $TREC^+$ T cells and TCR repertoire diversity has been reported [10]. Finally, the occurrence of GVHD [5], which likely impairs thymic function, can also affect long-term T cell immune reconstitution. All of these indications point to a progression of the immunodeficiency over time in transplanted SCID patients in the absence of donor stem cell engraftment.

Immunologic and clinical consequences of the decline in thymopoiesis after HSCT in SCID patients are significant. As shown in a recent survey of the European registry, at last follow-up, 16% of SCID patients transplanted with HSCT from an HLA-identical donor and 18% of SCID patients transplanted with HSCT from an haploidentical parent had a T cell immunodeficiency (defined as T cell lymphocytopenia (<1000/μL) or defective in vitro antigen T cell proliferation) [1].

It is striking to note that NK cell reconstitution is not as good as T cell reconstitution. This observation has been made following both HSCT and gene therapy, suggesting that the capacity for expansion of NK cell precursors is reduced compared to T cells [2]. It is presently unknown whether incomplete NK cell reconstitution might have clinical consequences. By analyzing clinical manifestations of SCID patients transplanted >10 years ago, the only significant difference observed between patients who originally had NK^- SCID and those who had NK^+ SCID is the more frequent occurrence of chronic skin human papilloma virus (HPV) disease [11]. NK^- SCID patients exhibit a significantly lower NK cell count (median 45/μL) than NK^+ SCID patients, 10 years or more after HSCT (median 178/μL). However, within the former group, patients with or without chronic human papillomavirus (HPV) disease do not differ for NK cell counts and function [11].

Another consequence of the lack of donor stem cell engraftment is the typical absence of donor B cells. This finding shows that B cell precursors contained in the bone marrow inoculum have a limited capacity for differentiation into multiple B cell clones and potentially that B cells have a limited half-life. It is also possible that competition with the host B cell lineage in B^+ SCID or even pro-B cells in B^- SCID impairs donor B cell differentiation. The frequently defective donor B cell reconstitution therefore leads to persistent B cell deficiency in a majority of patients [2,12]. These patients require long-term immunoglobulin replacement therapy. This need is explained by either an absence of host B cells (B^- SCID) or the presence of defective B cells (B^+ SCID caused by ILγc or JAK-3 mutations). Although in the latter case, some B cell function can be preserved [12,13], approximately 80% of patients do require immunoglobulin replacement therapy. A minority of SCID patients has long-lasting functional B cell immunity associated with host B cells, and they are mostly patients who had SCID with normal B cells (IL-7Rα and CD3 deficiencies).

How can long-term immunity be improved in SCID patients? Boost HSCT from an HLA-identical sibling with coinfusion of mature T cells rapidly restores immunity with clinical benefit. This effect is much more unclear for patients transpanted with haploidentical donors, given the risk of thymus inefficacy. To avoid secondary loss of T cell responses, newly diagnosed SCID patients should be treated in a way so that functional HSCs, those able to give rise to T lymphocytes (as well as NK and B lymphocytes) are present or persist. Allogeneic HSCT preceded by myeloablation to ensure donor stem cell engraftment is an option. There are multiple examples of long-term survival of SCID patients who received an HSCT preceded by full myeloablation (high-dose busulfan and cyclophosphamide) and who have stable functional T cells [1,14,15]. However, such an approach carries a very high risk of lethal toxicity in HSCT recipients. It might thus be selectively used in patients with mild or no infection and in NK^+ SCID patients who are at higher risk of graft failure. Gene therapy, provided that early pluripotent progenitors can be efficiently transduced is another option.

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Population-Based Newborn Screening for Severe Combined Immunodeficiency

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Infants with SCID are healthy at birth, but die of recurrent, severe infections in infancy unless they are provided with a functional immune system [1,2]. HSCT and enzyme replacement (in the case of adenosine deaminase deficiency) have made this previously fatal set of diseases treatable [2-10]. Gene therapy is also promising despite occurrences of leukemia related to retroviral insertional mutagenesis in 1 early trial [11]. At least 15 genes have been identified that when mutated cause SCID; if a specific gene defect has been defined in a family, prenatal or neonatal mutation diagnosis makes possible immediate treatment. From such families we know that infants treated before 3.5 months of age have better survival, less morbidity, and lower treatment costs than those recognized only after the onset of serious infections [12,13]. Thus, SCID meets most of the criteria that have been put forward for inclusion of new diseases in universal newborn screening programs (Table 1) [14]. SCID is fatal if untreated, and currently most affected infants do not come to medical attention until they develop opportunistic infections. Although the precise incidence of SCID is not known, it is estimated to be about 1/50,000 births, similar to diseases such as galactosemia and biotinidase deficiency, which are currently included in newborn screening panels. Certain populations, such as Indians of Athabascan heritage, have an increased incidence of autosomal recessive SCID, but all ethnicities are at risk.

Definitive diagnosis and effective treatment of SCID with HSCT are widely established. The best outcome for SCID, as with many other conditions for which newborn screening is now done, is

Table 1. *Justifications for Universal Newborn Screening**

Proposed Screening Criteria	How SCID Meets Criteria
Disease is serious	SCID is fatal in first 1-2 years of life if untreated
Disease is not detectable by examination	Newborns with SCID appear healthy
Incidence supports screening	SCID incidence unknown, estimated to be around 1 in 50,000 births
Confirmative testing is well established	Low absolute T cell count, negative mitogen proliferation in vitro
Effective treatment exists	Allogeneic hematopoietic stem cell transplantation
Earlier treatment is better than delayed treatment	Best survival and long-term outcome when treated before infections occur
Diagnosis and treatment are available	Pediatric immunologists, pediatric immunodeficiency transplantation centers in every region
Screening is cost-effective	An inexpensive, high-throughput screening test could save many lives

*Refs: Chan and Puck, 2005 [15]; Watson et al., 2006 [14].

achieved if HSCT is performed very soon after birth, ideally before clinical presentation with infections and failure to thrive. Cost-effectiveness of screening depends on development of an appropriate low-cost, high-throughput screening test, preferably 1 that can be done with the dried blood spots currently collected by all newborn screening programs. Chan and Puck [15] published a successful dried blood spot assay based on quantitating TRECs, which are intracellular byproducts of successful T cell antigen receptor rearrangement in the thymus. Absence of TRECs can identify T-lymphocytopenic SCID infants regardless of their genotype. A theoretical cost-benefit analysis has supported SCID newborn screening if a sufficiently inexpensive and accurate test is used [16]. In addition to absence of TRECs, high interleukin-7 (IL-7) levels could become an alternative or second-tier maker for increasing specificity of SCID detection [17].

A further impetus for SCID screening is the recommendation by public health authorities of vaccination programs that mandate use of live, attenuated virus vaccines, such as the new multivalent antirotavirus vaccine (given at 6 weeks of age), as well as live vaccines against varicella and measles-mumps-rubella. These pose a danger to infants with SCID [18]. Through early diagnosis, newborn screening would protect immunocompromised infants from potentially severe disease by avoidance of exposure to vaccine-strain viruses.

Although screening tests are designed to be sensitive, so as not to miss true cases, specificity, or minimizing false positive results, is also important to keep programs affordable and maintain the trust of community health providers. Plans are needed for patients who will be screen positive and then have follow-up studies that are not diagnostic for SCID, but are not totally normal. The process of screening for T cell lymphopenia, while intended to identify SCID, would in addition identify complete DiGeorge syndrome and perhaps other conditions.

Current screening programs have networks of metabolic disease specialists, hematologists, etc., throughout their states who are always available to be contacted in addition to the primary care provider [19]. The definitive diagnosis and treatment for SCID will likewise require recruitment of clinical specialists to perform follow-up evaluations. A nationally coordinated workup for all infants with results deposited in a central databank would be a valuable resource for assessing the performance of screening as well as providing normative data and population-based rates of abnormal immune parameters.

The present therapy of choice for SCID is allogeneic HSCT from an HLA-matched sibling. For infants without a matched, related donor, alternative donors and cell types are being used, including T cell-depleted (TCD), haploidentical- related marrow or peripheral blood stem cells (PBSC); matched unrelated donor stem cells; and matched unrelated cord blood. Currently, experimental gene therapies are being refined and may become widely available for SCID patients in the future. Despite overall improving outcomes of HSCT for SCID in many centers [8,20,21], questions about optimal treatment remain to be worked out with the aid of more complete data. There is no current consensus regarding a single optimal HSCT protocol for a very young, presymptomatic SCID infant without an HLA-matched sibling donor. Delayed or incomplete immune reconstitution can leave patients requiring lifelong intravenous immunoglobulin (IVIG), remaining at risk for lethal viral infections, and having poor nutritional status and autoimmunity. Sequellae of chemotherapy include infertility; osteopenia; endocrine, renal, and pulmonary dysfunction; and impaired tooth development. GVHD is not beneficial in HSCT for SCID, and can lead to organ dysfunction and autoimmunity. Collaborative studies based on information in shared registries could be used to weigh these factors and arrive at best practices for treatment of very young presymptomatic infants with SCID to be identified by newborn screening.

In conclusion, SCID newborn screening is being pursued with enthusiasm. By diagnosing SCID presymptomatically, newborn screening is anticipated to bring about early treatment to improve survival rates. Test methodologies need optimizaton and screening programs will need to be integrated with plans for definitive diagnosis and management. Definition of the true incidence of SCID, clarification of genetic versus environmental contributions to phenotypes and discovery of additional gene defects causing SCID are anticipated.

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